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Review

Amnesic shellfish poison

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Abstract

Amnesic shellfish poisoning (ASP) is caused by consumption of shellfish that have accumulated domoic acid, a neurotoxin produced by some strains of phytoplankton. The neurotoxic properties of domoic acid result in neuronal degeneration and necrosis in specific regions of the hippocampus. A serious outbreak of ASP occurred in Canada in 1987 and involved 150 reported cases, 19 hospitalisations and 4 deaths after consumption of contaminated mussels. Symptoms ranged from gastrointestinal disturbances, to neurotoxic effects such as hallucinations, memory loss and coma. Monitoring programmes are in place in numerous countries worldwide and closures of shellfish harvesting areas occur when domoic acid concentrations exceed regulatory limits. This paper reviews the chemistry, sources, metabolism and toxicology of domoic acid as well as human case reports of ASP and discusses a possible mechanism of toxicity.

Keywords: Amnesic shellfish poisoning; Domoic acid; Neurotoxin; Pseudonitzchia multiseries

Contents

Abbreviations: ASP, amnesic shellfish poisoning; i.p., intraperitoneal; i.v., intravenous; s.c., subcutaneous; NOAEL, no observed adverse effect level; LOAEL, lowest observable adverse effect level; HPLC, high performance liquid chromatography; GC-MS, gas chromatography coupled to mass spectroscopy; ELISA, enzyme linked immunosorbent assay; RIA, radio-immunoassay; BBB, blood brain barrier; bw, bodyweight; TSH, thyroid stimulating hormone; T_4, thyroxine.

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1. Introduction

A major human poisoning incident occurred in 1987 when over 150 people in Canada became acutely ill after ingesting mussels contaminated with an unknown toxin. The outbreak resulted in the hospitalisation of 19 individuals and the death of 4 elderly people. The clinical effects observed in many of the affected individuals included memory loss and for this reason the condition was termed amnesic shellfish poisoning (ASP). The chemical, which causes ASP, was subsequently identified as domoic acid, an amino acid produced by some species of phytoplankton. Domoic acid can be bio-concentrated by shellfish and thus, can then enter the human food chain. Following the 1987 outbreak of ASP, the Canadian authorities imposed an action limit of 20 μg domoic acid/g shellfish tissue. Programmes to monitor the domoic acid concentrations in shellfish are in place in many countries worldwide and numerous incidents of domoic acid contamination have been recorded in a wide variety of shellfish species.

This paper reviews the chemistry, sources, metabolism, and toxicology of domoic acid as well as human case reports of ASP and discusses a possible mechanism of toxicity.

2. Chemistry and analysis of domoic acid

The principal toxin responsible for amnesic shellfish poisoning (ASP) is domoic acid, an amino acid belonging to the kainoid class of compounds (Wright et al., 1990a). Domoic acid is produced by a number of marine organisms (Ohfune and Tomita, 1982) and ten isomers of domoic acid (isodomoic acids A to H and domoic acid 5′ diastereomer) have been identified in marine samples (Fig. 1, Maeda et al., 1986; Wright et al., 1990a, b; Wright and Quilliam, 1995; Zaman et al., 1997). Isodomoic acids are minor constituents relative to domoic acid and the isomers are not always present in shellfish contaminated with ASP (Table 1, Zhao et al., 1997). Domoic acid can be converted to isodomoic acids when exposed to ultra violet light or heat (Wright et al., 1990a; Wright and Quilliam, 1995; Ravn, 1995). Therefore, it has been suggested that domoic acid may be the product of biosynthesis, which is subsequently converted to isodomoic acids under appropriate environmental conditions (Wright et al., 1990a).

Table 1
Relative proportions of domoic and isodomoic acids detected in shellfish extracts

Compound	Mussel/%[a]	Razor clam/%[a]	Anchovy/%[a]
Domoic acid	82	73	90
Isodomoic acid D	8	21	7
Isodomoic acid E	3	6	3
Isodomoic acid F	1	nd	nd
Domoic acid 5′-diastereomer	6	nd	nd

[a] These proportions have been estimated by the authors of this review by measuring the peak heights in chromatogram figures from Zhao et al. (1997). nd not detected.

Domoic acid and its isomers are water-soluble and do not degrade under ambient temperatures or when exposed to light in sterile saline solution (Johannessen, 2000). However, it has been shown to decompose under acidic conditions (50% domoic acid loss in 1 week at pH 3) (Quilliam et al., 1989). It is not known if storage in vinegar also degrades domoic acid when it is present in shellfish.

Domoic acid possesses three carboxyl groups and a secondary amino group (Fig. 1) The pK_a values for the carboxyl groups are 2.10, 3.72, 4.97 and for the amino group is 9.82 (Piñeiro et al., 1999) thus, domoic acid can exist in a range of charged states (−3 to 1) depending on pH. At neutral pH all four groups are charged but if the pH is lowered the overall charge is reduced as the carboxyl groups become successively protonated.

Numerous methods have been developed to measure domoic acid. Some are sufficiently sensitive

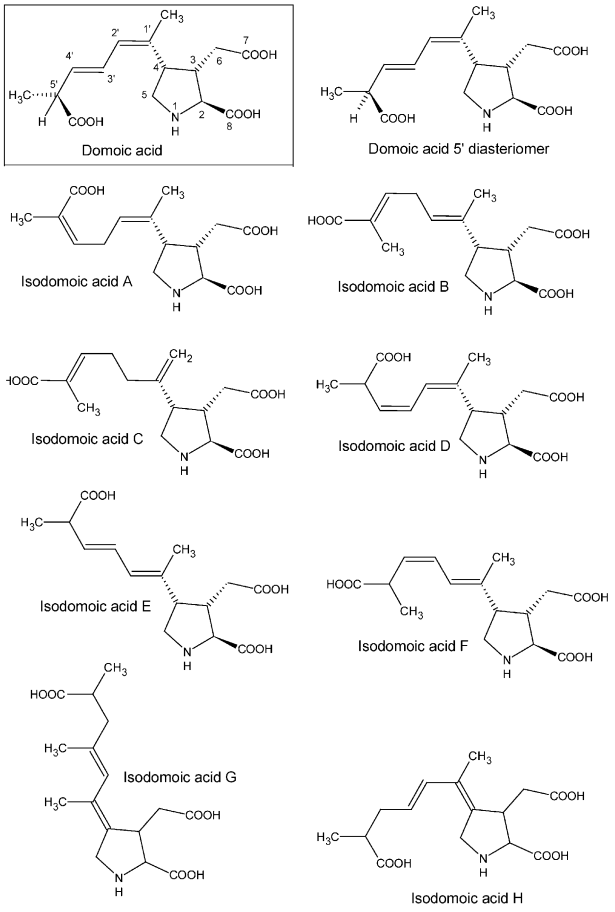

Fig. 1. Chemical structures of domoic acid its 5′ diastereomer and its isomers, isodomoic acids A–H.

to measure concentrations of domoic acid in samples of shellfish, phytoplankton and seawater as well as blood, urine and faecal samples from humans and animals. Analytical methods used to detect domoic acid are summarised in Table 2.

2.1. Sources and accumulation of domoic acid in shellfish

Domoic acid was originally identified from the macro red algae, *Chondria armata,* in Southern Japan (Takemoto and Daigo, 1958). However, following the 1987 outbreak of ASP in Canada, marine diatoms of the genus *Pseudo-nitzschia* were also shown to produce domoic acid (Perl et al., 1990).

Pseudo-nitzschia spp. are widely distributed across the world in sea waters of both warm and cold climates. There are seasonal variations in phytoplankton blooms with numbers increasing in spring and autumn when there is heavy rainfall and an abundance of nutrients (Bates et al., 1989). In addition, low wind and reduced water currents allow phytoplankton to accumulate in warmer surface waters, whilst periods of sunshine allow these 'standing stocks' to undergo rapid growth and form blooms (Trainer et al., 1998). Numerous strains of *P. nitzschia* are known to produce domoic acid including *P. multiseries*, *P. pseudodelicatissima* and *P. australis* (Hay et al., 2000) . However, production of domoic acid varies greatly with strain and is thought to be increased in response to environmental stresses, such as temperature change (Ramsey et al., 1998). Although warmer sea temperatures (14–17 °C) tend to be associated with increased domoic acid production (Walz et al., 1994), some strains have adapted to growth in cooler waters e.g. *P. seriata* produces high concentrations of domoic acid at 4 °C (Lundholm, 1994).

Domoic acid has been shown to accumulate in a wide variety of shellfish species such as types of cockles (*Cerastoderma edule*), crabs (*Cancer magister*), furrow shell (*Scrobicularia plana*), mussels (*Mytilus edulis*), razor clams (*Siliqua patula*) and scallops (*Pecten maximus*) (Wekell et al., 1994; Rhodes et al., 1998; Vale and Sampayo, 2001). Shellfish accumulate domoic acid either by direct filtration of the plankton or by feeding directly on contaminated organisms and thus, concentrations are highest in the digestive glands compared with other tissues. Rates of accumulation vary between different species of shellfish (Vale and Sampayo, 2001; Hay et al., 2000). Little is known about possible metabolism of domoic acid in shellfish (Vale and Sampayo, 2001) but studies have shown that there are marked differences in the rate of elimination (depuration) of domoic acid between shellfish species (Hay et al., 2000). For example, it has been shown that 50% of the domoic

Table 2
Some representative analytical methods for the detection and measurement of domoic acid in a range of matrices

Method	Matrices analysed	LOD (μg/g)[a]	Reference
Rodent bioassay	Mussels	~	Lawrence (1989)
HPLC-UV	Mussels	1	Lawrence et al. (1991)
HPLC-MS	Anchovy Razor clam Mussel Crab Serum Urine Faeces	0.1	Lawrence et al. (1994)
HPLC-fluorescence	Mussels	0.006	James et al. (2000)
HPLC-fluorescence	Phytoplankton	0.001	Sun and Wong (1999)
GC-MS	Mussel	1	Pleasance et al. (1990)
TLC	Mussel		Quilliam et al. (1998)
CE	Mussel Razor clam Anchovy	0.15	Zhao et al. (1997)
ELISA	Urine Serum Milk	0.2 μg/ml, 0.25 μg/ml, 10 μg/ml	Smith and Kitts (1994)
ELISA	Mussels Scallops Oysters Phytoplankton	0.038	Garthwaite et al. (1998)
ELISA	Mussel Crab Oyster	0.04	Kawatsu et al. (2000)
Receptor binding assay	Mussel extract	0.002 μg/ml	van Dolah et al. (1997)

[a] LOD (limits of detection) are in μg/g unless otherwise indicated.

acid content was eliminated from blue mussels (*Mytilus edulis*) within 24 hours (Novaczek, 1992) whereas, it took 86 days for a comparable proportion of domoic acid to be eliminated from razor clams (*Siliqua patula*) (Horner et al., 1993). Some fish species such as anchovies and mackerel have also been shown to accumulate domoic acid but the levels are much lower compared with those found in shellfish (Vale and Sampayo, 2001).

Studies on the effects of frozen storage and cooking on domoic acid concentrations in shellfish are limited but suggest that the overall levels of domoic acid are reduced by these processes (Villac et al, 1993; Hatfield et al, 1995; Leira et al, 1998).

2.2. Toxicology of domoic acid

Early toxicology studies on ASP were limited by the availability of large quantities of purified domoic acid. Therefore, many of these studies used small group sizes, shellfish extracts of unknown or estimated domoic acid concentrations and parenteral routes of administration to maximise the potency of the test material. In addition, many of these studies have reported clinical symptoms and behavioural responses as an indicator of the toxicity of domoic acid rather than more objective toxicological end-points. Very few studies have examined the effects of oral administration of domoic acid. Studies that have used low group numbers and poorly-defined dosing materials are highlighted.

2.3. Absorption, distribution, metabolism and excretion

Very limited data are available on the absorption, distribution, metabolism and excretion of domoic acid. No studies have investigated quantitatively the absorption of domoic acid. However, rodent studies by Iversen and co-workers have compared behavioural responses after i.p. and oral administration of domoic acid. No observable adverse effect levels (NOAELs) of 1 and 28 mg/kg bw and lowest observable adverse effect levels (LOAELs) (scratch behaviour) of 2 and 35 mg/kg bw were reported after i.p. and oral administration domoic acid, respectively (Iverson et al., 1989). In a further experiment, i.p. and oral administration of mussel extract containing domoic acid gave NOAELs of 2 and 60 mg/kg bw and LOAELs (seizures) of 4 and 80 mg/kg bw, respectively (Iverson et al., 1989). The appreciable differences in the effects observed after i.p. and oral administration suggest that domoic acid is poorly absorbed from the gut. However, the small group sizes used in these experiments ($n=1$ or 2/dose) and the subjective measures of effect do not allow a more quantitative assessment of bioavailability. In both studies domoic acid was almost entirely excreted in the faeces lending support to the suggestion that domoic acid is not well absorbed. Likewise, Truelove et al. (1996) reported that only 2% of the dose was excreted in the urine of rats administered domoic acid ($n=10$) by oral gavage (5 mg/kg bw/d). Similar findings were also reported by Truelove et al. (1997) in a study of cynomolgus monkeys ($n=3$) administered with a sub-toxic dose of domoic acid by oral gavage for 32 days. Mean urinary excretion of domoic acid was 4, 6 and 7% of the daily dose for each animal (Truelove et al., 1997).

Once absorbed, domoic acid appears to undergo little metabolism. In a study in rats ($n=8$), approximately 75% of a dose of ^{3}H-labelled domoic acid administered by i.v. injection was excreted unchanged in the urine within 160 min suggesting that it is not significantly metabolised prior to rapid elimination. However, in this study, the remaining radiolabel eluted earlier during liquid chromatography than domoic acid suggesting that some metabolism to compounds of greater hydrophilicity may have occurred (Suzuki and Hierlihy, 1993).

A study by Truelove and Iverson (1994) investigated the distribution and excretion of domoic acid in rats and monkeys. Domoic acid was administered by i.v. injection to rats (500, 1000 μg/kg bw, $n=2$ and 4, respectively) and cynomolgus monkeys (50 μg/kg bw, $n=4$) and a number of pharmacokinetic parameters were measured. Approximate half-lives of 20 and 110 min, apparent volumes of distribution of approximately 300 and 180 ml/kg and apparent clearances of approximately 10 and 1 ml/min/kg for domoic acid were reported for rats and monkeys, respectively. Although the pharmacokinetic parameters are derived from studies where small numbers of animals were used they suggest that domoic acid is well distributed into the body water in both species, as may be expected for a hydrophilic compound, and is rapidly eliminated. The data are too limited to draw conclusions on the possible species differences in the pharmacokinetics of domoic acid. The mechanism of urinary excretion of domoic acid has been investigated in rats by Suzuki and Hierlihy (1993). In this study, the total clearance (9.21 ml/min/kg) and renal clearance (9.88 ml/min/kg) of domoic acid were similar to that of inulin (7.50 and 8.10 ml/min/kg, respectively) but lower than that of *p*-aminohippuric acid (33.17 and 35.71 ml/min/kg, respectively). The renal clearance of domoic acid was unaffected by administration of probenecid (an inhibitor of active transport of organic acids from the blood). Thus, the authors suggest that the clearance of domoic acid from plasma is primarily by glomerular filtration. In contrast, the results of a small study by Robertson et al. (1992) showed that pre-treatment of rats with probenecid prior to i.p. administration of domoic acid resulted in an increase in the domoic acid plasma concentration (417%) compared with rats with normal renal function. This study suggests a role for active transporters in elimination of domoic acid in the kidney.

The transfer of domoic acid across the blood brain barrier (BBB) has been examined in a study in rats (Preston and Hynie, 1991). The transfer of ^{3}H-labelled domoic acid (12 μg/kg bw) across the BBB in rats was compared with that of ^{3}H-labelled sucrose, a compound that crosses the BBB by diffusion poorly, following i.v. administration. Transport constants (K_i) for the diffusion of domoic acid and sucrose into the brain were not significantly different in the brain regions analysed: frontal cortex, striatum, hippocampus, occipital cortex, diencephalon, cerebellum and pons-medulla (mean $K_i = 1.7\times10^6$ and 1.1×10^6 ml/g/s for domoic acid and sucrose, respectively) suggesting that domoic acid permeates through the BBB slightly more rapidly than sucrose and therefore, appears not to cross via a specific transport carrier. This conclusion was supported by the finding that the K_is were also unchanged by co-administration of a large amount non-radiolabelled domoic acid (0.5 mg/kg bw) (Preston and Hynie, 1991).

2.4. Toxicity studies in rodents

In an early study examining the acute toxicity of domoic acid by Iverson et al. (1989), a mussel extract containing domoic acid of an estimated concentration was serially diluted and administered by i.p injection to small groups of mice ($n = 3$/dose). This limited study showed that domoic acid was acutely toxic with an approximate LD_{50} of 2.4 mg domoic acid/kg bw. This LD_{50} value together with the NOAEL of 0.59 mg domoic acid/kg bw observed in this study, based on no observable clinical responses, suggested a steep dose-response curve. A similar value for the LD_{50} of 3.6 mg domoic acid/kg bw was reported by Grimmelt et al. (1990) in a study that also used small groups of mice ($n = 3$/dose) and i.p. administration of contaminated mussel extract. In both the studies the authors reported clinical symptoms such as scratching, tremors and seizures at both lethal and sub-lethal doses of extract (Iverson et al., 1989; Grimmelt et al., 1990). Iverson et al. (1989) also reported these clinical responses after oral administration of extracts to small numbers of mice ($n = 1$ or 2/dose) but much higher doses were required (35–60 mg domoic acid/kg bw). The behavioural responses to administration of domoic acid were examined in more detail in a limited study by Tasker et al. (1991). In this study, mice were administered mussel extract by i.p. injection over a range of doses (3.4–430 μg/animal, $n = 4$/dose). At lower doses hypoactivity was reported then sedation, rigidity, scratching and head weaving, loss of postural control, tremors, convulsions and death with increasing doses (Tasker et al., 1991). The authors suggest that such marked clinical responses were indicative of neurotoxicity.

The neuropathology in rodents administered with domoic acid was examined by Iverson et al. (1989) and indicated that domoic acid was a potent neurotoxin. Dose-related lesions in the brains of mice and rats were found after i.p., and to a much lesser extent, after oral administration of mussel extracts containing domoic acid. Oedema in the hypothalamus and hypothalamic arcuate nucleus and neuronal degeneration in the CA 3 region, and to a lesser in CA 1 and 4 regions, of the hippocampus were reported (Iverson et al., 1989) Similar neuropathology was consistently reported in other studies after i.p. administration of contaminated mussel extracts (Glavin et al., 1990) or i.p. (Strain and Tasker, 1991) or i.v. (Bruni et al., 1991) administration of domoic acid to mice and i.p. (Tryphonas et al., 1990a; Sobotka et al., 1996) or i.v. (Nakajima and Potvin, 1992) administration of domoic acid to rats. In these studies, doses of domoic acid in the range of approximately 1–7 mg/kg bw induced this characteristic neuropathology but this was not evident at lower doses of domoic acid.

The hippocampus is the area of the brain involved in functional memory and the effect of domoic acid on functional memory has been assessed in rodent studies using a behavioural endpoint (completion of a water maze task). Petrie et al. (1992) reported that domoic acid (2 mg/kg bw) administered by i.p. injection significantly impaired the ability of mice to remember how to navigate a water maze. Similarly, spatial learning and memory was also impaired in rats ($n = 5$/dose) administered with domoic acid (1.5 and 3 mg/kg bw) by i.p. injection (Kulmann and Guilarte, 1997). Clayton et al. (1999) studied the effects on functional memory of domoic acid (0, 1 or 2 mg/kg bw) administered by i.p injection to mice ($n = 12$/dose) as a single dose or as a repeated dose administered every 48 h on four occasions. Functional memory was affected by the single but not the repeat dosing regime. The authors suggest that domoic acid impairs functional memory but that effects are not observed after repeat dosing as alternative processes in the brain may accommodate for the initial memory loss. Effects on functional memory have also been reported in other studies following intraventricular injection of domoic acid (Sutherland et al., 1990; Nakajima and Potvin, 1992).

LD_{50} values have been reported for new born mice after parenteral administration of domoic acid. Xi et al. (1997) reported LD_{50} values in neonatal rats after i.p. administration as 0.25 mg/kg bw for animals at postnatal day (PND) 2 ($n = 20$/dose) and 0.7 mg/kg bw at PND 10 ($n = 15$/dose). When compared with adult LD_{50} figures, the lower LD_{50} value for neonatal rats suggests that they may be much more sensitive to domoic acid induced toxicity. This suggested neonatal sensitivity to domoic acid was supported by Wang et al. (2000) who reported an LD_{50} of 0.33 mg/kg bw after s.c. administration of domoic acid to mice at PND 7 mice ($n = 17$/dose). In this study, paralysis was induced in 11/17 and

17/17 mice following 0.33 and 0.42 mg domoic acid/kg bw. Respiratory failure, haemorrhaging, neuronal degeneration and stereotypic movement were also observed. Histopathological examination suggested that the behavioural changes may have been associated with domoic-acid induced spinal cord damage rather than brain lesions in these young animals (Wang et al., 2000).

A single short-term study of the effects on rodents of repeated exposure to low doses of domoic acid has been conducted. In this study, Truelove et al. (1997) administered domoic acid (0.01 or 5 mg/kg bw/d) by oral gavage to male and female rats (both $n = 10$/dose) for 64 days. No clinical abnormalities were observed during the course of treatment and there were no differences in haematology, clinical chemistry or histopathology between the treatment groups and a control group.

The effect of foetal exposure to domoic acid was explored in one study, however the effects reported were inconsistent. Domoic acid (0–2 mg/kg bw) was administered daily by i.p. injection to pregnant rats ($n = 9$–15/dose) from gestational day 7–16 and the mothers and their offspring were examined on gestational day 22 (Khera et al., 1994). Domoic acid treatment at doses of 1.75 and 2 mg/kg bw/d resulted in death in 6/12 and 6/9 mothers, respectively but all the animals survived to the end of the study in the lower dose groups. A reduction in the number of live foetuses at term was noted at doses of $\geqslant 0.5$ mg/kg bw/d compared to the control group but the number of deaths was not increased in a dose-dependent manner. A dose-dependent increase in the number of fetuses with visceral or skeletal anomalies was reported but this effect was not statistically significant (Khera et al., 1994).

The effects of domoic acid on thyroid function have been examined in two rodent studies. In the first study, statistically significant increases in thyroid stimulating hormone and T_4 concentrations were reported 1 h after i.p administration to rats of 1, but not, 0.5 mg domoic acid/kg bw compared with controls. However, after 2 h both doses led to significant decreases in T_4 concentrations (Arufe et al., 1995). Increased T_4 and TSH levels were also observed in a study by Alfonso et al. (2000) after i.p. administration of domoic acid (1 mg/kg bw) to rats. Although neither study established the duration of the effects on TSH and T_4, the authors suggest that they may be mediated by the actions of domoic acid on neuroendocrine regulation of thyroid hormones.

2.5. Toxicity studies in primates

Similar neurotoxic effects to those observed in rodents have been reported in studies of non-human primates. In a study by Tryphonas et al. (1990b), five cynomolgus monkeys were administered with single doses of domoic acid by i.p. (4 mg/kg bw) or i.v. (0.025, 0.05, 0.2 or 0.5 mg/kg bw) injection. Domoic acid administered by i.p. injection caused severe vomiting followed by hypothermia, acute pulmonary oedema and death within 4 h in the animal. Effects such as gagging and vomiting were also observed in all the animals following i.v. administration of domoic acid with increasing severity of clinical symptoms with dose. However, all four animals recovered within 3 h of dosing. At post-mortem, neutrophil vacuolation and hyperchromasia were noted in the hippocampus, hypothalamus, area postrema and the inner layers of the retina only in the animals treated with the 0.5 mg/kg bw (i.v.) and 4 mg/kg bw (i.p.) doses of domoic acid. Although the data are very limited, in a subsequent analysis Tryphonas et al. (1990c) suggest that the induction of emetic effects after parenteral administration of domoic acid may be via a neuroexcitory mechanism possibly by neuronal excitation of the area postrema, an area of the brain that lies outside the BBB that has been associated with emesis (Borison et al., 1984). In addition, because clinical symptoms were observed at all the doses but neuropathology was only evident at the highest dose the authors suggest that doses <0.5 mg/kg bw (i.v.) may be neuroexcitatory but that doses $\geqslant 0.5$ mg/kg bw (i.v.) are excitotoxic (Tryphonas et al., 1990c). Similar clinical symptoms were reported in a larger study of cynomolgus monkeys administered with single doses of domoic acid of 0.25 ($n = 2$), 0.5 ($n = 5$), 1 ($n = 6$) 1.25 ($n = 1$), 1.5 ($n = 2$), 2 ($n = 2$) and 4 mg/kg bw ($n = 2$) by i.v. injection (Scallet et al., 1995). Domoic acid induced severe vomiting and scratching in all the animals and the time to onset of the symptoms was dose-related. Doses of domoic acid $\geqslant 1.0$ mg/kg bw resulted in death by respiratory failure within 2–7 h in 4/7 animals with recovery in the remaining animals. On post-mortem, localised lesions in the CA 2 region of the hippocampus were evident in animals that had received doses of 0.5 and 1.0 mg domoic acid/kg bw, whilst higher doses caused widespread damage to pyramidal neurons and axon terminals in the CA 1–4 regions of the hippocampus (Scallet et al., 1995). In an additional study of the tissues collected utilising specialised histochemistry, neuronal degeneration was observed not only in the hippocampus but also in structures of the thalamus (Schmued et al., 1995).

Two studies using very low group numbers of animals have examined the effects of oral administration of domoic acid in cynomolgus monkeys. In the first study, seven cynomolgus monkeys were administered single doses of either domoic acid (0.5, 5 or 10 mg/kg bw) or four mussel extracts containing domoic acid (5.6, 5.9, 6.5 or 6.6 mg/kg bw) by oral gavage (Tryphonas et al., 1990d). No effects were observed at the lowest dose. In the other treated animals, symptoms including anorexia, salivation, retching, vomiting, diarrhoea and fatigue were evident in all the animals but the range, severity and time until onset of the responses were variable. The inconsistent nature of the symptoms may have been due

to the protective effect of vomiting resulting in a significantly reduced absorbed dose in some animals with emetic responses. Autopsy at 44 days showed neuronal shrinkage and degeneration in the CA 3 and CA 4 regions of the hippocampus in some animals (Tryphonas et al., 1990d). No clinical symptoms were observed in a repeat dose study of cynomolgus monkeys administered lower doses of domoic acid (0.5 mg/kg bw/d for 15 days and 0.75 mg/kg bw/d for a further 15 days) by oral gavage ($n = 3$). No significant changes in body weight, food or water consumption, or haematology, clinical chemistry or brain histology were observed (Truelove et al., 1997).

3. Genotoxicity

The structure of domoic acid contains a butadiene moiety, which raises the possibility for the formation of DNA-reactive expoxides in vivo. Data on the possible genotoxicity of domoic acid are limited to one in vitro study, which examined the mutation frequency in V79 Chinese hamster lung cells exposed to domoic acid (87 or 174 μM) with and without a rat-derived metabolising system. Domoic acid did not increase mutation frequency in this cell line as measured by thioguanine or ouabain resistance, sister chromatid exchange or micronucleus assays (Rogers and Boyes, 1989). These limited data suggest that, domoic acid itself is not reactive to DNA, as may be expected from its structure, nor does it give rise to DNA-reactive metabolites. No published studies have examined the in vivo mutagenicity or carcinogenicity of domoic acid.

3.1. Human case reports of ASP

Data on the toxicological effects of domoic acid in humans have been derived from the single documented outbreak of ASP, which occurred in Canada in 1987 (Perl et al., 1990). Acute illness characterised by gastrointestinal and unusual neurological symptoms was reported in the people affected, which was traced to ingestion of blue mussels (*Mytilus edulis*). Cases of this condition, were clinically defined as the occurrence of gastrointestinal symptoms within 24 h and/or of neurologic symptoms within 48 h from consumption of mussels. Approximately 150 reports of this illness were received, although only 107 individuals fulfilled the clinical definition of the illness and of these the most common gastrointestinal symptoms were vomiting (76%), abdominal cramps (50%) and diarrhoea (42%) and the most common neurological symptoms were severe headache (43%) and loss of short-term memory (25%). The illness was particularly severe in 19 individuals who were hospitalised and 12 individuals with particularly severe symptoms (e.g. seizures, coma, profuse respiratory secretions or unstable blood pressure) required treatment in an intensive care unit. Of these patients 8/12 were $\geqslant 65$ years whilst 4/12 had pre-existing illnesses which included insulin-dependent diabetes, renal disease and hypertension. Three patients died in hospital 11-24 days after consumption of mussels (Perl et al., 1990).

It was established that the mussels which had been ingested had been contaminated with domoic acid from a bloom of the phytoplankton *Pseudonitzschia f. multiseries* in the Port Edward Island region, where the mussels had originated (Perl et al., 1990). Concentrations of domoic acid in mussels collected from the implicated region after the outbreak ranged from 1.9–5.2 mg/g tissue (Perl et al., 1990). Domoic acid was not detected in samples of blood or cerebrospinal fluid of 17 patients that were tested. However, there was an interval of at least 2 days between ingestion of the mussels and this analysis, which may have been long enough for domoic acid to have been excreted from the body (Perl et al., 1990).

Estimates of exposure were made from analysis of leftover mussels collected from households or restaurants identified in 9 cases of poisoning (as well as 1 case where no clinical symptoms were observed). Concentrations ranged from 31–128 mg domoic acid/100 g mussel tissue and from these results estimates of the amount of domoic acid ingested were calculated for each patient on the basis of either the amount of mussels the patient could recall consuming or an average serving size (Perl et al., 1990). The amount of domoic acid ingested ranged from 60–290 mg/patient in those patients that became ill. The estimates of the amount of domoic acid ingested together with the some of clinical symptoms reported and treatment received are shown in Table 3 and indicate a dose-related increase in the severity of the symptoms reported. This apparent increasing severity of clinical symptoms with dose of domoic acid confirms the association between domoic acid and the illness. From this rudimentary estimation of the dose, it would appear that ingestion of as little as 60 mg domoic acid/person, approximately equivalent to 1 mg domoic acid/kg bw/d, is sufficient to induce gastrointestinal illness. Similarly, ingestion of 270 mg domoic acid/person, approximately equivalent to 4.5 mg domoic acid/kg bw results in neurological effects. The advanced age of many of the severely affected individuals may suggest that poor renal function of this population sub-group may be a factor in the severity of the domoic acid-related toxicity (Perl et al., 1990).

A report by Teitelbaum et al. (1990) examined the neurological dysfunction in 14 subjects that fulfilled the ASP case definition and had been severely affected during the 1987 outbreak. All the patients had exhibited unsteadiness, generalised weakness, confusion and disorientation within the first 48 h after consuming

contaminated mussels, 5 patients had seizures and 13 patients had altered states of consciousness ranging from agitation to coma with most patients recovering within 72 h. Examination of the electroencephalograms of 7 patients taken within 1 week of poisoning indicated that all had moderate to severe generalised slowing of background activity. This evaluation was made on all 14 patients, 4 months later and showed that 3 patients had normal activity but 11 patients still exhibited mild to moderate disturbance of background activity. When psychological tests were made 4–6 months later, 12 of the patients had an anteriograde memory disorder and severely affected individuals suffered from retrograde amnesia. Teitelbaum et al. (1990) also examined the neuropathology of 4 subjects that had died within 4 months of consuming contaminated mussels during the outbreak [an additional death was identified after the report by Perl et al. (1990)]. Neuronal necrosis or loss and astrocytosis were prominent in the CA1 and CA3 regions of the hippocampus and amygdaloid nucleus in all 4 subjects. Lesions were also evident in the thalamus of 2 subjects and in the subfrontal cortex of 3 subjects (Teitelbaum et al., 1990). Cendes et al. (1995) reported severe bilateral hippocampal sclerosis on autopsy of an elderly patient 3 years after the ASP outbreak who had been severely affected by, but had survived, the illness.

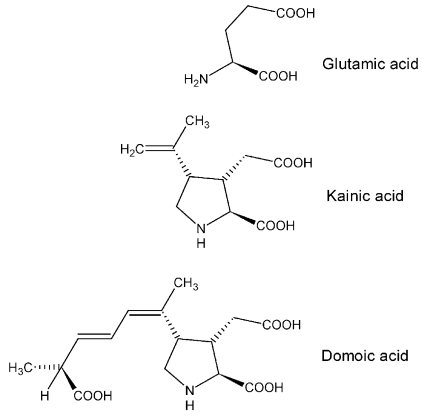

Fig. 2. Structural similarities of glutamic, kainic and domoic acids.

It has been suggested that ASP may have been responsible for an outbreak of human illness in 21 individuals with gastrointestinal and/or mild neurological symptoms after consumption of razor clams in Washington State (USA) in 1991. Although razor clams from the area were found to be contaminated with domoic acid, the illness was not confirmed as ASP and the poisoning incident was not well reported and was unsubstantiated (Todd, 1993).

3.2. Mechanism of toxicity

The possible mechanisms of action of domoic acid toxicity have been explored by in vitro studies. The structural similarity of domoic acid to glutamic acid and, in particular, kainic acid (Fig. 2), a compound known to bind in the brain to a group of the glutamate receptor family (referred to as the kainate receptor subtype) prompted domoic acid-glutamate receptor binding studies. Domoic acid was found to have particularly strong affinity for subclasses of the kainate receptor (see Table 4) (Hampson et al., 1992; Lomeli et al., 1992; Hampson and Manalo, 1998). When compared to domoic acid, the affinities of isodomoic acids to kainate receptors are significantly lower (IC_{50} of domoic acid of 4.9 nM compared with 28, 1400 and 940 nM for isodomoic acids D, E and F, respectively) thus, the structure of the isodomoic acid side chains has a profound effect on the receptor binding affinities (Hampson et al., 1992).

Kainate receptors are widely distributed in the mammalian brain but are particularly concentrated in the CA 1-3 regions of the hippocampus in rodents (Wisden and Seeburg, 1993), CA 2 and CA 3 regions in non-

Table 3
Estimated exposure, clinical symptoms and treatment of patients who ingested mussels during the 1987 Canadian ASP outbreak. Adapted from Perl et al. (1990)

Patient	Age	Estimated weight of mussels consumed (g)[a]	Domoic acid in mussels (mg/100g)	Estimated domoic acid consumed (mg)	Clinical symptoms and treatment			
					GI[b]	Memory loss	Hospitalised	ICU
Control	60	35	52	20	–	–	–	–
1	72	120	52	60	+	–	–	–
2	62	150	45	70	+	+	–	–
3	70	15	52	80	+	–	–	–
4	61	300	31	90	+	–	–	–
5	67	160	68	110	+	–	–	–
6	71	360	31	110	+	–	–	–
7	74	400	68	270	+	+	+	–
8	68	225	128	290	+	+	+	+
9	84	375	76	290	+	+	+	+

[a] Estimates of the amount consumed were made from average restaurant serving when amount consumed was unknown.
[b] GI refers to gastrointestinal symptoms e.g. vomiting, diarrhoea or abdominal cramps.

Table 4
Affinities of domoic, kainic and glutamic acids for subtypes of kainate receptors

Compound	GluR5[a]/nM	GluR6[a]/nM	GluR7[a]/nM	KA1[b]/nM
Domoic acid	2	9	37	11
Kainic acid	73	36	62	2
Glutamic acid	290	1080	2190	480

Affinities are expressed as K_as for kainic acid (equilibrium constant for binding of kainic acid) and K_is for domoic and glutamic acids (equilibrium constant for inhibition of kainic acid binding).
[a] Lomeli et al. (1992).
[b] Hampson and Manalo (1998).

human primates (Carroll et al., 1998) and CA 3 region in humans (Porter et al., 1997). The high affinity of domoic acid to kainate receptors and the apparent co-localisation of these receptors and the sites of domoic acid induced damage evident in the brains of rodents and non-human primates and humans strongly suggests that domoic acid-kainate receptor interactions mediate the toxic response. This binding of domoic acid to glutamate receptor subtypes appears to stimulate neuronal firing. In studies by Debonnel et al. (1989; 1990), localised administration of domoic or kainic acid to rat hippocampus resulted in increased neuronal firing in the CA 1 region and to a greater extent in the CA 3 region with the potency of domoic acid significantly exceeding that of kainic acid. In addition, Dakshinamurti et al. (1991) reported increased neuronal firing together with seizures in rats after microinjection of domoic acid into the CA 3 hippocampal region. Domoic acid was also shown to induce excitation in cultured rat hippocampal neurons (Stewart et al., 1990). Thus, domoic acid binding to glutamate receptors elicits an excitatory response in neuronal cells both in vitro and in vivo.

The precise mechanism of neuronal stimulation leading to tissue damage in these regions of the brain is not well understood. However, in vitro experiments by Nijjar (1993) showed that an influx of calcium ions (Ca^{2+}) into cells occurred in brain tissue slices exposed to domoic acid. Xi and Ramsdell (1996) also reported increased cytosolic Ca^{2+} levels in hippocampal pyramidal neurons exposed to domoic acid. In addition, increases in *c-fos* levels, a gene upregulated in response to increased Ca^{2+} concentrations, following domoic acid treatment of CA1 pyramidal cells was reported by Peng et al. (1996) and Peng and Ramsdell (1997). In a study monitoring real time intracellular Ca^{2+} concentrations in cultured neurons, Berman et al. (2002) reported that concentrations increased in response to domoic acid treatment and was correlated with neuron loss. The authors showed that the increase in Ca^{2+} concentrations was due to activation of glutamate receptors and voltage sensitive calcium channels and a reversal of the sodium/calcium ion exchanger (Berman et al., 2002). Thus, it appears domoic acid binding to glutamate receptors in specific regions of the brain leads to excitation of neurons, giving rise to an influx of Ca^{2+} resulting in a failure to maintain intra-cellular ion homeostasis and neuronal cell death. Recently, Ananth et al. (2003a,b,c) have suggested that increased nitric oxide synthesis in neurones may also contribute to the neurotoxicity of domoic acid.

3.3. Regulation of domoic acid concentrations in shellfish

After the 1987 ASP outbreak, the Canadian authorities imposed an action limit for domoic acid in mussels of 20 μg domoic acid/g mussel flesh, which when exceeded would result in closure of shellfish harvesting areas. This action limit was derived from a retrospective estimation of level of domoic acid in mussels, which had caused illness in some consumers during the ASP outbreak (200 μg domoic acid/g of mussel flesh) and incorporation a 10-fold safety factor (Waldichuk, 1989). The action limit employed by Canada has been adopted elsewhere and is the limit enforced in the European Union, United States of America, New Zealand and Australia for domoic acid in a variety of shellfish species.

4. Discussion

Domoic acid is a neurotoxin produced by sea-water phytoplankton of the *Pseudo-nitzschia* group. During phytoplankton blooms, which are stimulated by environmental conditions, elevated concentrations of domoic acid can be produced. Shellfish feeding on the phytoplankton can bio-concentrate domoic acid leading to a potentially serious health hazard for people consuming the contaminated shellfish. Indeed, an outbreak of poisoning in a human population in Canada occurred in 1987 when mussels contaminated with domoic acid were consumed. The outbreak resulted in approximately 150 cases of poisoning, 19 of whom were hospitalised, and 4 deaths. Neurological effects including amnesia were observed in some patients and as a result the condition was termed amnesic shellfish poisoning.

Data on the absorption, distribution, metabolism and excretion of domoic acid are very limited. The available information suggests that following ingestion, domoic acid is not well absorbed from the gut, undergoes little metabolism and is excreted rapidly in rodents and non-human primates. Studies in rodents indicate that domoic acid permeates across the BBB poorly. However, despite the limited transfer across the BBB, the brain is the primary site of domoic acid-induced toxicity.

Data on the toxicology of domoic acid are also limited. Many studies have used small numbers of animals and in some cases shellfish extracts contaminated with domoic acid have been used instead of the pure compound. However, the principal toxicological effects

found in these studies are reported consistently and when taken together the available information show that domoic acid is acutely toxic in the central nervous system. In studies of rodents and non-human primates, characteristic neurobehavioral effects such as scratching, tremors and seizures with the addition of emesis in non-human primates were induced on administration of domoic acid as either a semi-purified shellfish extract or as a pure substance. The emetic effects in non-human primates were induced both after parenteral and oral administration of domoic acid. It is therefore, unclear whether emesis is a result of direct action of domoic acid on the gastrointestinal tract or a result of a centrally mediated response possibly by activation of the area postrema, an area of the brain that lies outside the BBB that has been associated with emesis. It is possible that both mechanisms may act in tandem. A comparison of the oral doses of domoic acid that gave rise to behavioural effects in studies of rodents and non-human primates suggest that non-human primates may be more sensitive to domoic acid induced toxicity (e.g. a NOAEL of 5 mg domoic acid/kg bw in rats versus a LOAEL of 5 mg domoic acid/kg bw in primates for behavioural responses). However, the data are too limited to assess the relative sensitivities quantitatively.

A study of neonatal rodents suggests that they may be significantly more susceptible to domoic acid induced toxicity than adults. This difference in susceptibility may be a reflection of the relatively underdeveloped BBB in neonates when compared with adults.

A limited study of the potential teratogenicity of domoic acid produced inconsistent results and no firm conclusions could be made on the basis of this study.

A limited in vitro study suggests that domoic acid is not DNA-reactive nor is it metabolised to a DNA-reactive compound.

The similar neurobehavioral effects of domoic acid observed in studies in rodents and non-human primates and reported in the human poisoning cases indicate that domoic acid is excitotoxic in a range of mammalian species. The histopathological examination included in studies of rodents, non-human primates and from human cases indicates domoic acid is also neurotoxic and causes neuronal degeneration and necrosis in some areas of the brain, particularly in specific regions of the hippocampus. Lesions induced in the hippocampus are consistent between rodent and non-human primates studies and parallel those found during autopsy of human patients that died as a result of the 1987 ASP outbreak. The hippocampus is an area of the brain involved in memory and therefore damage to this area is consistent with the amnesic effects reported in some patients.

The high affinity of domoic acid to a subgroup of glutamate receptors and the apparent co-localisation in specific regions of the brain of these receptors and domoic acid induced necrosis strongly suggests a role for these receptors in domoic acid toxicity. However, the precise mechanism of toxicity is unclear. It is possible that binding of domoic acid to these receptors induces hyperexcitation of neurones in the hippocampus, which leads to excessive accumulation of intracellular calcium ions resulting in disruption of cellular ion homeostasis and cell death.

Following the human outbreak of ASP in 1987 an action limit of 20 μg domoic acid/g shellfish flesh was introduced in Canada and subsequently in other parts of the world. When the action limit is exceeded harvesting of shellfish is prohibited. The limit is based on a retrospective estimate of the quantity of domoic acid in mussels that resulted in human illness in the 1987 Canadian ASP and incorporates a ten-fold safety factor. Although this action level may not be considered a toxicologically based safety limit, the lack of reports of ASP cases would suggest that this action level has prevented further outbreaks of ASP on the scale of the 1987 Canadian incident.

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